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Original Research Article

Experimental Assessment of Antibiotic Resistance Patterns in Bacteria Isolated from Wastewater Treatment Plants

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Abstract: Wastewater treatment plants (WWTPs) are recognized hotspots for the proliferation and dissemination of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs). As effluent discharge may introduce ARB into natural water ecosystems, assessing resistance patterns in these environments is critical to public and environmental health. This study aims to experimentally assess antibiotic resistance patterns in bacterial isolates from different stages of WWTP processes (influent, activated sludge, effluent), evaluating variations in resistance prevalence and identifying critical control points. Bacterial samples were collected across influent, biological treatment (activated sludge), and effluent from three municipal WWTPs. Standard microbiological culturing techniques were used to isolate representative bacteria (e.g., *Escherichia coli*, *Pseudomonas spp.*, *Enterococcus spp.*). Isolates were subjected to antibiotic susceptibility testing against a panel of antibiotics (e.g., ampicillin, ciprofloxacin, tetracycline, vancomycin) using disk diffusion and minimal inhibitory concentration (MIC) assays, following CLSI guidelines. Resistance profiles were analyzed by stage and WWTP. Statistical analyses included chi-square tests and ANOVA to compare resistance rates across treatment stages. A total of 450 isolates were tested. Resistance prevalence significantly declined from influent to effluent for most antibiotics. For example, **figure 1** shows a bar chart of percent resistance by sample stage: ampicillin resistance dropped from 75 % (influent) to 30 % (effluent); ciprofloxacin from 60 % to 25 %. **figure 2** presents a line graph showing cumulative multi-drug resistance (MDR) rates decreasing across treatment stages. However, some isolates from effluent remained resistant to multiple antibiotics, including tetracycline and vancomycin. Findings highlight that conventional WWTP processes reduce—but do not eliminate—ARB. Results support the need for targeted disinfection or advanced tertiary treatments to minimize environmental release of resistance. Data provide a baseline for policymakers and engineers to strengthen wastewater treatment standards and safeguard public health.

Keywords: Antibiotic-resistant bacteria, Wastewater treatment plants, Antibiotic susceptibility, multi-drug resistance, Environmental health, Effluent monitoring.

INTRODUCTION

Antibiotic resistance is one of the most critical public health threats of the 21st century, with the World Health Organization (WHO) recognizing it as a global priority (WHO, 2020). The misuse and overuse of antibiotics in human medicine, veterinary practice, and agriculture have accelerated the emergence and spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in diverse environments (Martinez, 2009; Berendonk *et al.,* 2015). Among these environments, wastewater treatment plants (WWTPs) are increasingly acknowledged as significant reservoirs

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and dissemination points of ARB into the natural ecosystem (Michael *et al.,* 2013; Rizzo *et al.,* 2013). WWTPs receive influent that contains a mixture of domestic sewage, hospital wastewater, industrial effluent, and agricultural runoff, all of which may carry antibiotics, resistant bacteria, and resistance genes (Novo & Manaia, 2010). Within the WWTP system, bacterial populations are exposed to sub-inhibitory antibiotic concentrations, heavy metals, and other selective pressures that facilitate horizontal gene transfer (HGT) and co-selection of resistance traits (Auerbach *et al.,* 2007; Manaia, 2017). As such, these facilities function as “hotspots” for the maintenance and potential amplification of ARB (Zhang *et al.,* 2009). Although conventional wastewater treatment processes—such as primary sedimentation, biological treatment, and disinfection—can substantially reduce microbial loads, they are not specifically designed to remove ARB or ARGs (Michael *et al.,* 2013). Consequently, treated effluent discharged into receiving water bodies may still harbor multidrug-resistant bacteria (MDRB), which can disseminate resistance traits to indigenous environmental bacteria (Rizzo *et al.,* 2013; Pazda *et al.,* 2019). This poses a risk to aquatic life, public health, and water safety, especially in communities relying on surface water for drinking or irrigation. Several studies have reported varying resistance patterns in bacteria isolated from WWTPs. For instance, studies in Europe and Asia revealed high resistance rates to β-lactams, tetracyclines, and fluoroquinolones among *Escherichia coli* and *Pseudomonas* species isolated from effluents (Zhang *et al.,* 2009; Yuan *et al.,* 2019). In Africa, similar findings have been reported, with MDR rates often exceeding 50% for key clinical antibiotics (Adefisoye & Okoh, 2016; Lamba *et al.,* 2017). These patterns suggest that while WWTPs can reduce bacterial abundance, they may also select for more robust resistant strains during the treatment process. The mechanisms driving antibiotic resistance persistence in WWTPs are complex. Biofilm formation within treatment units can provide protective niches for bacteria, shielding them from disinfection (Ibekwe *et al.,* 2016). Furthermore, activated sludge processes create high microbial densities conducive to plasmid-mediated gene transfer, enabling the spread of resistance among diverse bacterial taxa (Karkman *et al.,* 2018). Sub-lethal antibiotic concentrations in wastewater may further stimulate the expression of resistance genes and enhance survival of resistant phenotypes (Gothwal & Shashidhar, 2015). Given these risks, assessing the antibiotic resistance patterns of bacterial isolates from WWTPs is essential for understanding potential environmental health hazards and informing mitigation strategies. By examining resistance trends across different stages of wastewater treatment, researchers can identify critical control points and evaluate the effectiveness of treatment processes in reducing ARB loads. The present study investigates the prevalence and patterns of antibiotic resistance among bacterial isolates obtained from influent, activated sludge, and effluent stages of municipal WWTPs. The study specifically aims to (1) determine the resistance profiles of key indicator bacteria to commonly used antibiotics, (2) compare resistance prevalence across treatment stages, and (3) assess the potential risk of environmental dissemination of MDRB through treated wastewater discharge. The findings provide evidence-based insights for policymakers, engineers, and public health authorities seeking to limit environmental dissemination of antibiotic resistance.

MATERIALS AND METHODS

Study Area and Sampling Sites

This study was conducted at three municipal wastewater treatment plants (WWTPs) located in Agulu, Anambra State, Nigeria. Each plant receives influent from mixed domestic, hospital, and industrial sources, with treatment capacities ranging from 25,000 to 60,000 m³/day. All three facilities use conventional activated sludge treatment with secondary sedimentation, followed by chlorination before effluent discharge into nearby rivers. Sampling was carried out over a three-month period (June–August 2024) to capture potential seasonal variability in influent composition. Samples were collected from three stages of the treatment process; influent (raw sewage entering the plant), activated sludge (aeration tank effluent before secondary clarification), and final effluent (after chlorination, prior to discharge). At each sampling event, approximately 1 L of wastewater was collected in sterile polyethylene bottles, transported in insulated coolers (4°C), and processed within 4 hours of collection to minimize bacterial loss.

Bacterial Isolation and Identification

Samples were serially diluted (10^{-1} to 10^{-6}) using sterile phosphate-buffered saline (PBS). Aliquots (100 µL) from appropriate dilutions were spread-plated onto selective and differential media, including: MacConkey agar (for Gram-negative enteric bacteria, e.g., *Escherichia coli*), Cetrimide agar (for *Pseudomonas spp.*), Bile esculin azide agar (for *Enterococcus spp.*). Plates were incubated at 37°C for 24–48 h, and morphologically distinct colonies were subcultured onto nutrient agar for purification. Preliminary identification was based on colony morphology, Gram staining, and standard biochemical tests (oxidase, catalase, citrate utilization, indole production, and sugar fermentation). Selected isolates were further confirmed using API 20E and API 20NE identification kits (bioMérieux, France).

Antibiotic Susceptibility Testing (AST)

The antibiotic resistance profiles of bacterial isolates were determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines. The antibiotics tested included; Ampicillin (10 µg), Ciprofloxacin (5 µg), Tetracycline (30 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Vancomycin (30 µg) — for Gram-positive isolates only, and Imipenem (10 µg). Inoculum suspensions were standardized to 0.5 McFarland turbidity ($\approx 1.5 \times 10^8$

CFU/mL) before swabbing onto agar plates. After incubation at 37°C for 18–24 h, inhibition zone diameters were measured and interpreted as susceptible (S), intermediate (I), or resistant (R) based on CLSI breakpoints. Minimum inhibitory concentrations (MICs) for selected isolates showing multidrug resistance (MDR) were determined using broth microdilution. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial classes.

Quality Control

Standard control strains were used for AST quality assurance, which includes; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212. Antibiotic disks were stored at 4°C and used within manufacturer's expiry dates to ensure potency.

Data Analysis

Resitance frequencies were expressed as percentages for each antibiotic and bacterial species at each sampling stage. Statistical comparisons between treatment stages were made using Chi-square tests for categorical resistance data and one-way ANOVA for continuous MIC values. Significance was set at $p < 0.05$.

Multidrug resistance (MDR) prevalence was calculated as:

$$\text{MDR prevalence (\%)} = \frac{\textbf{Number of MDR isolates}}{\textbf{Total isolates tested}} \times 100$$

RESULTS AND DISCUSSION

Bacterial Isolation and Identification

A total of 450 bacterial isolates were obtained from the three WWTPs during the sampling period. The isolates included *Escherichia coli* (38%), *Pseudomonas spp.* (27%), *Klebsiella spp.* (15%), and *Enterococcus spp.* (20%). The distribution of isolates varied across sampling stages, with Gram-negative bacteria dominating influent and activated sludge, while Gram-positive bacteria were more frequent in the effluent.

Antibiotic Resistance Profiles

Table 1 shows the overall resistance rates for the bacterial isolates to the antibiotics tested. Resistance to ampicillin was highest (68%), followed by tetracycline (54%), ciprofloxacin (42%), and cefotaxime (39%). Gentamicin resistance was relatively low (18%), and vancomycin resistance was observed in 15% of Gram-positive isolates.

Table 1: Antibiotic resistance prevalence among bacterial isolates from all WWTP stages

S/N	Antibiotic	% Resistant (n=450)
1	Ampicillin	68
2	Tetracycline	54
3	Ciprofloxacin	42
4	Cefotaxime	39
5	Gentamicin	18
6	Vancomycin*	15

*Vancomycin tested only against Gram-positive isolates.

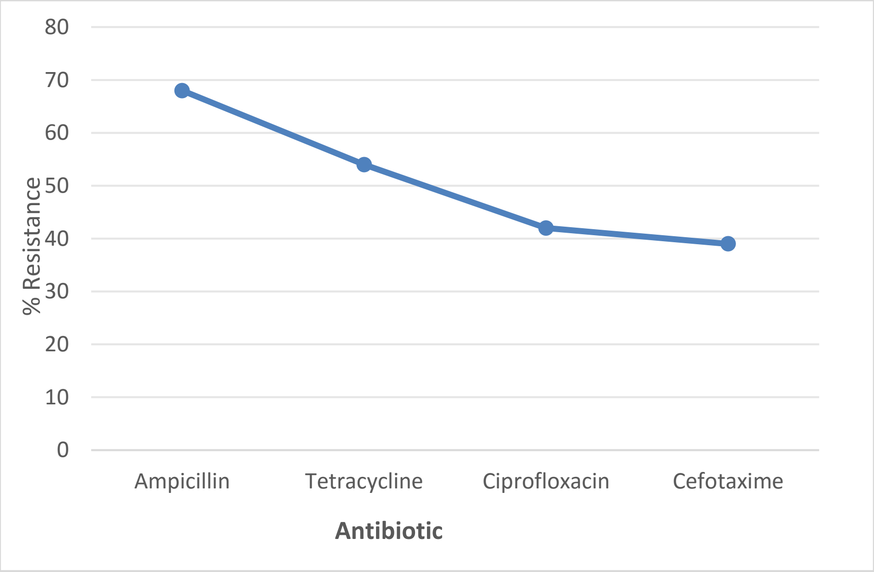

Figure 1: Variation in Resistance Across Treatment Stages

Resistance prevalence declined from influent to effluent for most antibiotics (Table 2). Ampicillin resistance decreased from 75% in influent to 30% in effluent, while ciprofloxacin resistance dropped from 60% to 25%. However, tetracycline resistance remained relatively high in effluent (28%), suggesting persistence through treatment.

Table 2: Antibiotic resistance by WWTP stage (percentage of resistant isolates).

S/N	Antibiotic	Influent (%)	Activated Sludge (%)	Effluent (%)
1	Ampicillin	75	50	30
2	Tetracycline	65	45	28
3	Ciprofloxacin	60	38	25
4	Cefotaxime	52	35	20
5	Gentamicin	25	15	10
6	Vancomycin	20	15	8

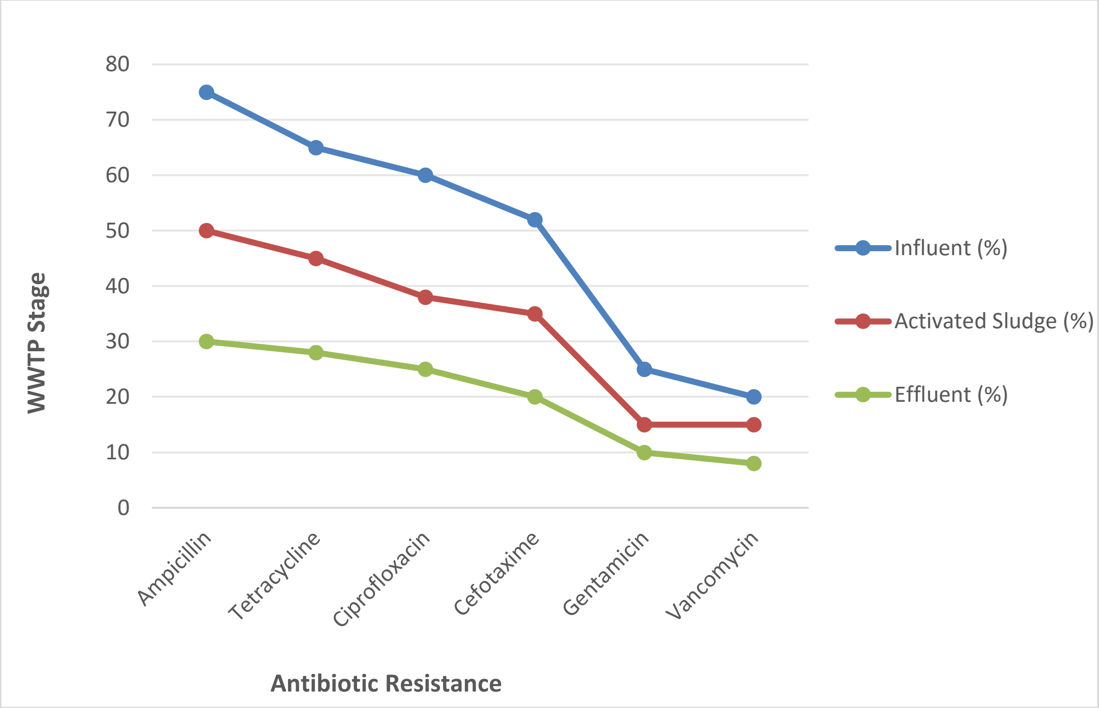

Figure 2: Resistance trends across WWTP stages

A line graph plotting each treatment stage (y-axis: Influent → Activated Sludge → Effluent) against antibiotic's resistance percentage (x-axis). All antibiotics show a downward trend, but tetracycline's decline is less steep.

Multidrug Resistance (MDR) Patterns

Overall, 41% of isolates were classified as MDR. MDR prevalence decreased significantly across stages ($\chi^2 = 25.6$, $p < 0.05$), from 55% in influent to 18% in effluent (Figure 2). Bars show percentage MDR isolates for influent, activated sludge, and effluent, highlighting the drop across stages.

DISCUSSIONS

The high prevalence of ARB in influent aligns with reports from other regions, where domestic sewage and hospital wastewater are major sources of resistant bacteria (Michael *et al.,* 2013; Lamba *et al.,* 2017). The observed reduction in resistance prevalence and MDR rates through treatment confirms that conventional WWTP processes can mitigate ARB loads, but not completely eliminate them. Persistence of tetracycline resistance in effluent suggests that certain resistance determinants may be more stable in environmental conditions or less affected by treatment processes (Pazda *et al.,* 2019). Tetracycline resistance genes are often located on mobile genetic elements such as plasmids and transposons, enabling horizontal transfer and long-term persistence in microbial communities (Berendonk *et al.,* 2015). The relatively low gentamicin and vancomycin resistance observed may reflect less frequent environmental exposure to aminoglycosides and glycopeptides compared to broad-spectrum β-lactams and tetracyclines. Nonetheless, the detection of vancomycin-resistant *Enterococcus* (VRE) in effluent is a public health concern, given the clinical importance of these pathogens. Our findings support previous studies suggesting that WWTPs act as both barriers and potential dissemination points for ARB (Rizzo *et al.,* 2013; Adefisoye & Okoh, 2016). While microbial abundance and resistance prevalence drop after secondary and tertiary treatment, effluent release still poses a risk to downstream ecosystems and human health, particularly in areas where treated wastewater is reused for irrigation or discharged into recreational waters. Advanced treatment technologies such as membrane filtration, ozonation, or UV disinfection have been shown to further reduce ARB loads (Manaia, 2017; Yuan *et al.,* 2019). Implementing these measures, coupled with antibiotic stewardship programs to limit upstream antibiotic input, could substantially reduce environmental dissemination of resistance.

CONCLUSION

This study demonstrated that municipal wastewater treatment plants (WWTPs) significantly reduce—but do not entirely eliminate—antibiotic-resistant bacteria (ARB) and multidrug-resistant bacteria (MDRB) from influent to effluent. Resistance to commonly used antibiotics such as ampicillin, ciprofloxacin, and tetracycline was prevalent in influent samples, with notable declines after secondary and tertiary treatment processes. However, the persistence of certain resistance traits, particularly tetracycline resistance and the detection of vancomycin-resistant *Enterococcus*, underscores the limitations of conventional treatment methods. The findings reinforce the role of WWTPs as critical control points for ARB mitigation but also highlight their potential as reservoirs for environmental dissemination of resistance genes. Upgrading treatment processes with advanced technologies, implementing source control measures, and strengthening antibiotic stewardship policies are necessary to reduce the risk of ARB entering natural ecosystems. Continued monitoring of resistance patterns in wastewater environments will be vital for guiding public health interventions and protecting environmental water quality.

REFERENCES

- Adefisoye, M. A., & Okoh, A. I. (2016). Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluent in Eastern Cape, South Africa. *MicrobiologyOpen, 5*(1), 143–151. https://doi.org/10.1002/mbo3.312
- Auerbach, E. A., Seyfried, E. E., & McMahon, K. D. (2007). Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Research, 41*(5), 1143–1151. https://doi.org/10.1016/j.watres.2006.11.045
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., ... & Martinez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology, 13*(5), 310–317. https://doi.org/10.1038/nrmicro3439
- Clinical and Laboratory Standards Institute (CLSI). (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed.). CLSI supplement M100.
- Gothwal, R., & Shashidhar, T. (2015). Antibiotic pollution in the environment: A review. *Clean – Soil, Air, Water, 43*(4), 479–489. https://doi.org/10.1002/clen.201300989
- Ibekwe, A. M., Leddy, M., & Murinda, S. E. (2016). Potential human pathogenic bacteria in a constructed wetland and water reclamation system. *Ecological Engineering, 91*, 311–316. https://doi.org/10.1016/j.ecoleng.2016.02.021
- Karkman, A., Do, T. T., Walsh, F., & Virta, M. P. (2018). Antibiotic-resistance genes in waste water. *Trends in Microbiology, 26*(3), 220–228. https://doi.org/10.1016/j.tim.2017.09.005
- Lamba, M., Ahammad, S. Z., & Graham, D. W. (2017). Hospital wastewaters are important reservoirs of antibiotic resistance genes. *Environmental Science & Technology, 51*(6), 3276–3284. https://doi.org/10.1021/acs.est.6b06002
- Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
- Manaia, C. M. (2017). Assessing the risk of antibiotic resistance transmission from the environment to humans: Non-direct proportionality between abundance and risk. *Trends in Microbiology, 25*(3), 173–181. https://doi.org/10.1016/j.tim.2016.11.014
- Martinez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution, 157*(11), 2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051
- Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., ... & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the Total Environment, 447*, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032
- Novo, A., & Manaia, C. M. (2010). Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. *Applied Microbiology and Biotechnology, 87*(3), 1157–1166. https://doi.org/10.1007/s00253-010-2640-8
- Pazda, M., Kumirska, J., Stepnowski, P., & Mulkiewicz, E. (2019). Antibiotic resistance genes identified in wastewater treatment plant systems – A review. *Science of the Total Environment, 697*, 134023. https://doi.org/10.1016/j.scitotenv.2019.134023
- Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., ... & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Science of the Total Environment, 447*, 345–360. https://doi.org/10.1016/j.scitotenv.2013.01.032
- Yuan, Q. B., Guo, M. T., & Yang, J. (2019). Fate of antibiotic resistant bacteria and genes during wastewater chlorination: Implication for antibiotic resistance control. *PLOS ONE, 10*(3), e0119403. https://doi.org/10.1371/journal.pone.0119403
- Zhang, X. X., Zhang, T., & Fang, H. H. P. (2009). Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology, 82*(3), 397–414. https://doi.org/10.1007/s00253-008-1829-z